

In the Claims

C 1
1. (twice amended) The method for determining the relative ratio of LDL to HDL or at least two different apolipoproteins in a biological sample comprising:

immersing into the sample a solid phase material having separately immobilized thereon at least first and second monoclonal antibody molecules immunoreactive with [a specific lipoprotein indicative of] LDL [or], HDL or VLDL or at least two different apolipoproteins, wherein the first and second monoclonal antibodies bind to either LDL, HDL or VLDL or to different apolipoproteins in a conformation and lipid content independent manner;

Sub D.
allowing the monoclonal antibody molecules time to bind to the [lipoprotein in the] LDL [and], HDL or VLDL or apolipoproteins in the sample;

removing the solid phase material containing the immobilized monoclonal antibody molecules;

determining the amount of LDL [and], HDL or VLDL lipoprotein or at least two different apolipoproteins bound by the immobilized monoclonal antibody molecules, and

comparing the amount bound which is specific for LDL [or], HDL or VLDL or each apolipoprotein in order to calculate the relative amounts of LDL [and], HDL or VLDL or apolipoproteins.

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2. (amended) The method of claim 1 wherein the antibody molecules immobilized on the solid phase material are immunoreactive with [a] lipoproteins selected from the group

C2 consisting of HDL[,] and LDL[, VLDL, and combinations thereof].

C3 3. (twice amended) The method of claim 2 wherein the [antibody is] antibodies to the HDL or LDL are selected from the group consisting of recombinant antibodies and antibody fragments.

C4 4. (amended) The method of claim 3, wherein the [antibody is] antibodies comprise the anti-LDL monoclonal antibody produced by the hybridoma cell line HB₃CB₃ ATCC designation number HB 11612.

Sub D2 5. (amended) The method of claim 3, wherein the [antibody is a] antibodies comprise recombinant anti-LDL RCB₃M₁D₄ ATCC designation number 69602.

C5 6. (twice amended) The method of claim 1 [wherein] further comprising determining the amount of lipoprotein or lipid associating with apolipoprotein [is determined] by staining of the material bound to the immobilized antibody using a lipid stain.

C6 9. (twice amended) The method of claim 6 further comprising measuring the amount of apolipoprotein or protein associated with lipid in the sample, further comprising the step of providing [a third antibody immunoreactive with apolipoprotein wherein the third] antibody [is] immunoreactive with the apolipoproteins coupled to a protein stain [and used to stain lipoprotein] , and staining the apolipoprotein or protein associated with lipid in the sample, [prior to immersing into the sample the immobilized first antibodies which then bind to the stained second antibody-bound apolipoprotein].

C7 12. (twice amended) A method of determining the relative concentration of at least

two different apolipoproteins in a biological sample comprising:

mixing in solution a first and second monoclonal antibody molecules each immunoreactive with a specific apolipoprotein into the sample, wherein at least one of the first and second monoclonal antibodies bind to different apolipoproteins in a conformation and lipid content independent manner;

allowing the monoclonal antibody molecules to bind to the apolipoprotein in the sample, immersing into the mixture third immobilized monoclonal antibody molecules immunoreactive with a second, distinct epitope of [an apolipoprotein] one of the apolipoproteins, allowing the third immobilized monoclonal antibody molecules to bind to the apolipoprotein,

detecting the presence of the apolipoprotein bound by both [both] one of the first and second monoclonal antibodies and the third immobilized monoclonal antibodies, and determining the amount of [each] apolipoprotein bound by both one of the first and second monoclonal antibodies and the third immobilized monoclonal antibodies.

13. (amended) The method of claim 12 wherein the apolipoprotein bound by one of the monoclonal antibodies in solution is apolipoprotein Apo B-100.

39. (amended) A method for determining the relative ratio of LDL to HDL in a biological sample comprising

(a) determining the amount of LDL in the sample by adding to the sample monoclonal antibody molecules immunoreactive with low density

lipoprotein and not cross-reactive with high density lipoprotein and determining the amount of low density lipoprotein;

(b) determining the amount of HDL in the sample by

adding to the sample monoclonal antibody molecules immunoreactive with high density lipoprotein and not cross-reactive with low density lipoprotein and determining the amount of high density lipoprotein; and

(c) determining the ratio of the amount of low density lipoprotein with the amount of high density lipoprotein, wherein at least one of the monoclonal antibodies to LDL and HDL bind in a conformation and lipid content independent manner.

40. (amended) A method for determining the relative ratio of VLDL to HDL in a biological sample comprising

(a) determining the amount of VLDL in the sample by

determining the amount of Apo C-III present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing monoclonal antibody specifically immunoreactive with Apo C-III,

contacting the anti-ApoC-III antibody reactive with Apo C-III with the biological sample to form complexes between the anti-ApoC-III antibody and the Apo C-III containing lipoprotein particles,

contacting the Pan B antibody with the biological sample containing the anti-ApoC-III

antibody bound to the Apo C-III containing lipoprotein particles,

separating the complexed Pan B-anti-ApoC-III antibody-lipoprotein particles from the biological sample, [and

determining] which is the amount of Apo C-III [associated with Apo B, which is the amount of Apo C-III] present in VLDL in the anti-Apo C-III anti-Apo B complexed material in the sample; and

(b) determining the amount of HDL in the sample by

determining the amount of Apo C-III present in the HDL in the sample by

providing Apo A-I monoclonal antibody specifically immunoreactive [specifically] with Apo A-I,

providing monoclonal antibody specifically immunoreactive with Apo C-III,

contacting the antibody reactive with Apo C-III with the biological sample to form complexes between the anti-Apo C-III antibody and the Apo C-III containing lipoprotein particles,

contacting the anti-Apo A-I antibody with the biological sample to form complexes with the anti-Apo C-III antibody-Apo C-III containing lipoprotein particles,

separating the complexed antibody-lipoprotein particles from the biological sample,

determining the amount of Apo C-III [associated with Apo A-I, which is the amount of Apo C-III] present in HDL in the anti-Apo C-III-anti-Apo A-I complexed material in the sample, and

determining the ratio of Apo C-III present in VLDL in the sample and Apo C-III present in HDL in the sample, which is the ratio of VLDL to HDL,

wherein the VLDL and HDL are measured in the same sample using immobilized anti-Apo A-I and anti-Apo B or anti-Apo C-III antibodies or measured by immunoprecipitation with the anti-Apo A-I and anti-ApoB antibodies or anti-Apo C-III antibodies in separate samples,

wherein at least one of the monoclonal antibodies bind to Apo AI, Apo B, or Apo CIII in a conformation and lipid content independent manner.

41. (amended) A method for determining the relative ratio of VLDL to HDL comprising

(a) determining the amount of VLDL in the sample by determining the amount of Apo E present in the VLDL in the sample by providing Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing monoclonal antibody which specifically binds to Apo E associated [predominantly] with VLDL,

contacting the antibodies reactive with Apo E associated with VLDL with the biological sample to form complexes between the anti-ApoE antibodies and Apo E containing particles,

contacting Pan B antibody with the biological sample containing the complexes between the anti-ApoE antibodies and ApoE containing particles to form complexes of anti-ApoB-anti-ApoE-ApoE containing particles, and

determining the amount of Apo E [associated with Apo B] in the complexes of anti-ApoB-anti-ApoE-ApoE containing particles, which is the Apo E present [predominantly] in VLDL in the sample;

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(b) removing the [complexed anti-Apo E:Pan B:Apo E] complexes of anti-ApoB-anti-ApoE-ApoE containing particles, either by binding [by immobilization] of the anti-Apo E antibodies to an immobilized surface or centrifugation of [complexed particles] sample to remove the complexes of anti-ApoB-anti-ApoE-ApoE containing particles;

and

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(c) determining the amount of HDL in the sample by
determining the amount of Apo E present in the HDL in the sample by
providing Apo A-I monoclonal antibody immunoreactive specifically with Apo A-I,
providing monoclonal antibody which binds to Apo E [predominantly] associated with HDL,

contacting the antibodies reactive with Apo E [to] with the biological sample to form complexes between the anti-ApoE antibodies and Apo E containing particles,

contacting Pan B antibody with the biological sample for form complexes of the anti-ApoE antibodies-ApoE containing particles-anti-ApoB,

determining the amount of Apo E [associated with Apo A-I, which is the amount of Apo E] present in HDL in the complexes of the anti-ApoE antibodies-ApoE containing particles-anti-ApoB in the sample, and

determining the ratio of Apo E present in VLDL in the sample and Apo E present in HDL in the sample which is the ratio of VLDL to HDL,

wherein at least one of the monoclonal antibodies bind to Apo B, Apo AI, or Apo E in a conformation and lipid content independent manner.

42. (amended) A kit for determining the relative ratio of VLDL to HDL comprising Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

monoclonal or recombinant antibody specifically immunoreactive with Apo C-III, and

monoclonal or recombinant Apo A-I antibody specifically immunoreactive with Apo A-I,

wherein at least one of the monoclonal or recombinant antibodies bind to Apo B, Apo AI, or Apo CIII in a conformation and lipid content independent manner.

43. (amended) The kit of claim 42 wherein the anti-Apo C-III or anti-A-I monoclonal or recombinant antibody molecules [specifically immunoreactive with a single specific lipoprotein or apolipoprotein] are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.

44. (amended) A kit for determining the relative ratio of VLDL to HDL comprising Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

monoclonal antibody which predominantly binds to Apo E associated with VLDL ,
monoclonal Apo A-I antibody specifically immunoreactive with Apo A-I, and
monoclonal antibody which predominantly binds to Apo E in HDL.

45. (amended) The kit of claim 44 wherein the anti-Apo E or anti-Apo A-I
monoclonal or recombinant antibody molecules [specifically immunoreactive with a single
specific lipoprotein or apolipoprotein] are selected from the group consisting of monoclonal
antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to
a stable, conformation independent epitope which is uninfluenced by the lipid content of the
lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.

46. (amended) A kit for determining the relative ratio of LPA-I and LPA-II
lipoprotein particles comprising
monoclonal or recombinant Apo-A-I antibody specifically immunoreactive with Apo A-I
lipoproteins in human plasma; and

monoclonal or recombinant Apo A-II antibody specifically immunoreactive with Apo A-
wherein the anti-Apo A-I or anti-Apo A-II monoclonal or recombinant antibody
molecules are selected from the group consisting of monoclonal antibodies, recombinant
antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation
independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein,
or lipid associated with a specific lipoprotein.